COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Susan Power of Cell & Molecular Technologies, Inc., Phillipsburg, New Jersey, United States of America, declare as follows:

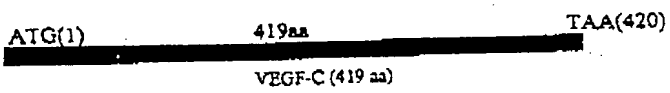
1. I am currently a Senior Molecular Biologist for Cell & Molecular Technologies, Phillipsburg, New Jersey. I have held this position since July, 1999. Prior to that appointment I was a post-doctorate research scientist, studying the molecular biology of retenoids and their role in limb bud development, at the University of Wisconsin, Madison, Wisconsin for two years. Prior to that appointment, I was a post-doctorate research scientist, studying the molecular biology of the transcription factor vHNF1 at the Pasteur Institute in Paris, France for four years. I received my Ph.D. in 1991 in Microbiology, from the National University of Ireland, Galway, Ireland.

2. The Patent Attorneys for Human Genome Sciences (HGS) requested that I perform the following experiments in order to determine whether the 350 amino acid form of VEGF-2 (corresponding to residues 70 to 419 of the 419 amino acid form of VEGF-2) fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells. They have also requested that all experiments that I conducted employ techniques routinely available by March, 1994. I have done this and the experiments I have conducted are described herein. Unless I state otherwise, all methods used herein were available prior to March, 1994.

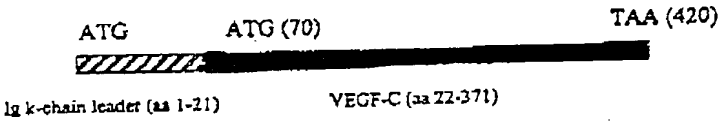
3. To determine whether the 350 amino acid form of VEGF-2 could be secreted from cells when attached to a heterologous signal sequence, I transfected eukaryotic cells with expression vectors encoding the 419 amino acid form of VEGF-2, or the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence. I grew the transfected cells under conditions to allow the cells to express the gene products encoded by the vectors. At various time points, I collected both cell lysates and culture medium and assayed for the presence of VEGF-2, in order to determine if the cells were secreting VEGF-2. The presence of VEGF-2 in either the cell lysates or culture medium was determined by Western Blot analysis of the samples using a polyclonal antibody to VEGF-2, which recognizes both the precursor form and the processed form of VEGF-2

4. The design of the expression vectors used in the study is as follows:

419 amino acid form of VEGF-2 (followed by a STOP codon at position 420):



350amino acid form of VEGF-2 linked to a heterologous signal sequence (followed by a STOP codon at position 420):



5. The nucleotide sequences encoding the 419 and the 350 amino acid forms of VEGF-2 were obtained directly from the American Type Culture Collection (ATCC). ATCC Deposit No. 97149 contains the nucleotide sequence encoding the 419 amino acid form of VEGF-2. ATCC Deposit No. 75698 contains the nucleotide sequence encoding the 350 amino acid form of VEGF-2. The nucleotide sequence encoding the 419 amino acid form of VEGF-2

was engineered to be flanked by an Eco RI site at the 5'end and a Not I site at its 3'end. The second construct contained the nucleotide sequence encoding the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, Ig k-chain leader signal sequence, a recognized signal sequence which was available as of March, 1994, and was engineered to contain a Bam HI site at its 5'end and a Not I site at its 3' end. The sequence of each of these constructs was confirmed to be correct and is detailed in Appendix I, attached hereto.

6. Each VEGF-2 construct was subcloned into the expression vector pCMV-I (see attached plasmid map, attached hereto as Appendix II), so that the VEGF-2 sequences were under the control of a CMV-I promoter, a promoter routinely used as of March, 1994. The 419 amino acid form of VEGF-2 was subcloned into the Eco RI/Not I sites of pCMV-I, while the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, was subcloned into the Bam HI/Not I sites of pCMV-I (see plasmid map, Appendix II).

7. The two VEGF-2 constructs were transiently transfected in duplicate, using the lipofectin method, comparable methods were routinely used as of March, 1994, into the Human Embryonic Kidney cell line, HEK-293 tsA-0, a cell line which was also routinely used as of March, 1994. As a control for transfection efficiency, each construct was co-transfected with the vector pCMV-β-gal. The efficiency of transfection was determined by β-gal staining 48 hours after transfection. As a negative control the vector pCMV-I without an insert was transfected in parallel.

8. The transfection design is as follows:
10 dishes transfected with: pCMV-I-VEGF-419;
10 dishes transfected with: pCMV-I-signal sequence-VEGF-350;
10 dishes transfected: pCMV-I ;
2 dishes transfected with: pCMV-I-VEGF-419 + pCMV-β-gal; and
2 dishes transfected with: pCMV-I-signal sequence-VEGF-350 + pCMV-β-gal.

9. After transfection, DMEM medium containing 3% serum was added to the cells. Aliquots of cell extracts and conditioned medium were prepared from each transfection at: T_0 hours, T_{16} hours, T_{24} hours, T_{48} hours and T_{72} hours, in duplicate.

10. At the time of harvesting the cells and medium were treated as follows:
Medium: Harvested medium was concentrated 3 fold using Centricon 10 concentrator devices. One volume of 2 x PAGE loading dye was added to each sample.
Cell Extracts: The cells were harvested by trypsinization and collected by centrifugation. The cell pellet was resuspended and lysed in 250µl of 1x PAGE loading buffer.

11. To determine the transfection efficiency, dishes transfected with the pCMV-β-gal construct were fixed and stained for β-gal activity 48 hours after transfection. All dishes were found to have the same percentage of transfected cells (70%).

12. Each protein sample was subjected to Western analysis as outlined below. Prior to loading on to a 12% (w/v) Tris-Glycine SDS-Polyacrylamide gel, the samples were boiled for 5 minutes and cooled on ice. The two end lanes of each gel contained the appropriately sized molecular weight markers to estimate the migration rate of proteins predicted to run in the 16 to 85 kDa size range. The samples were electrophoresed according to standard conditions.

13. Following electrophoresis, the samples were transferred to a PVDF membrane. Each membrane was blocked by a one hour incubation in phosphate buffered saline (PBS) containing 3% Bovine Serum Albumin (BSA). The blot was then incubated at 4°C in PBS containing 0.1% BSA and 500ng/ul of purified rabbit anti-VEGF-2 antibody, a polyclonal antibody which recognizes the precursor form and the processed form of VEGF-2. After three 5 minute washes in PBS containing 0.1% Tween, the blot was incubated for 1 hour in PBS containing 0.1% BSA and a 1:3000 dilution of Goat Anti-Rabbit IgG

Horse Radish Peroxidase (HRP) conjugated antibody. The blot was washed three times for 5 minutes in PBS containing 0.1% BSA. The blot was developed with 2ml/blot of ECL detection reagent (obtained from Amersham) for one minute and then exposed directly to Polaroid films for approximately 5 seconds.

14. The result of the experiment is shown in Figure 1, attached hereto. The samples included in the figure are as follows:

Immunoblot analysis of VEGF derivatives transiently expressed in HEK293T cells

Lane	Pellet/Supernatant	350aa -signal / 419aa	T (h) post-transfection
Gel 1			
1	P	419	24
2	S	419	24
3	P	350-signal	24
4	S	350-signal	24
5	P	350-signal	24
6	S	350-signal	24
7	P	negative control	24
8	S	positive control	48
Gel 2			
9	P	419	48
10	S	419	48
11	P	419	48
12	S	419	48
13	P	350-signal	48
14	S	350-signal	48

15	P	350-signal	48
16	S	350-signal	48
17	P	negative control	
18	S	negative control	
Gel 3			
19	P	419	72
20	S	419	72
21	P	419	72
22	S	419	72
23	P	350-signal	72
24	S	350-signal	72
25	S	350-signal	72
26	P	350-signal	72

15. The Western Blot analysis indicates that a doublet of approximately 30kDa was present in the medium collected from the transfection of both the 419 amino acid form of VEGF-2 and the 350 amino acid-VEGF-2 signal sequence constructs (see Figure 1). The secreted protein was visible beginning at 16 hours after transfection. The secreted product from cells containing the 419 amino acid construct and the 350 amino acid-VEGF2 signal sequence construct are approximately the same size.

AND I declare that all the statements made in this Declaration are of my own are true in every particular, and that all statements made on information and belief are believed to be true.

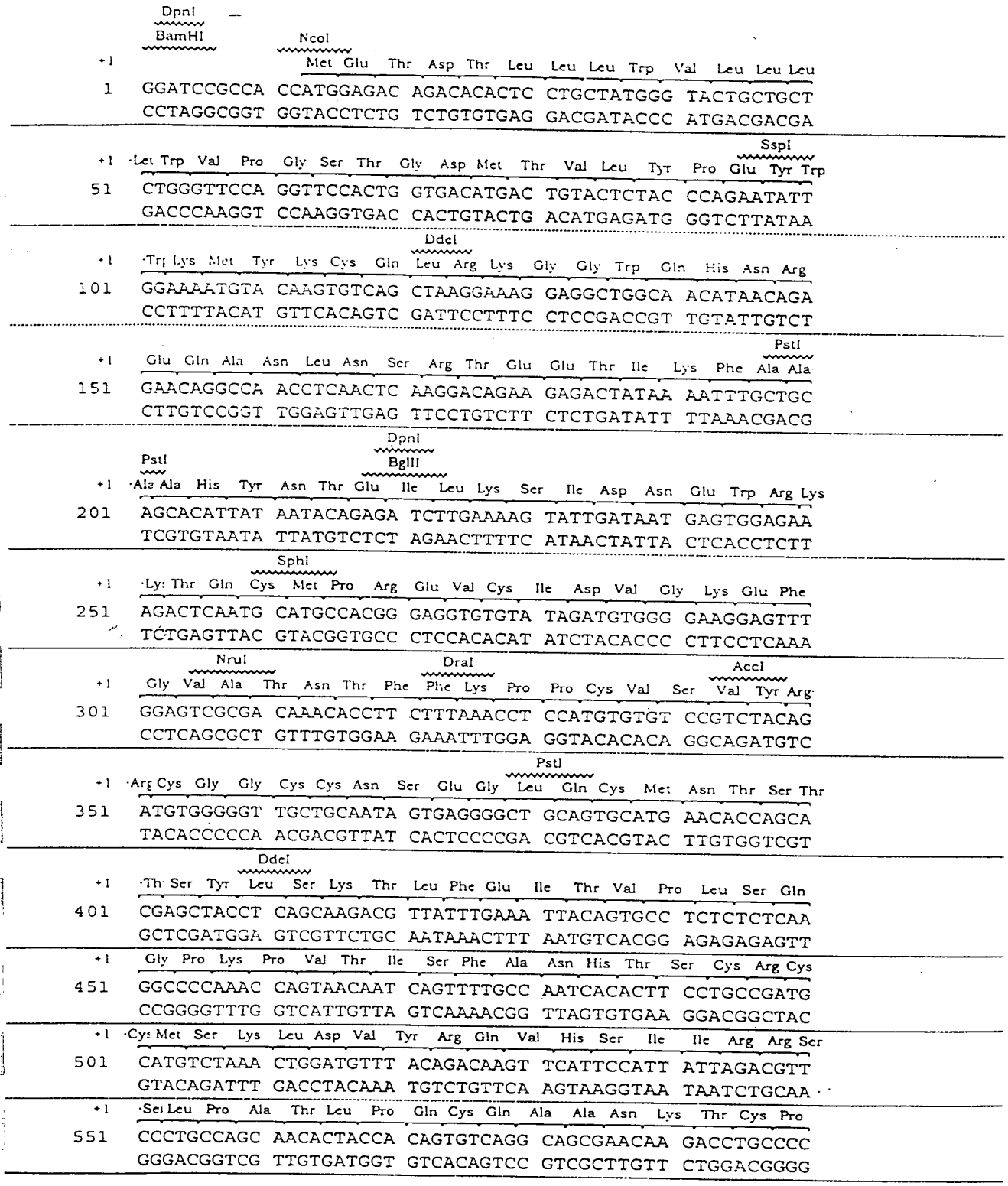
Sworn by the said Susan Power, Susan Power at Phillipsburg, New Jersey, on this 13th day of December 2000; before me Maryann White.

Notary Public

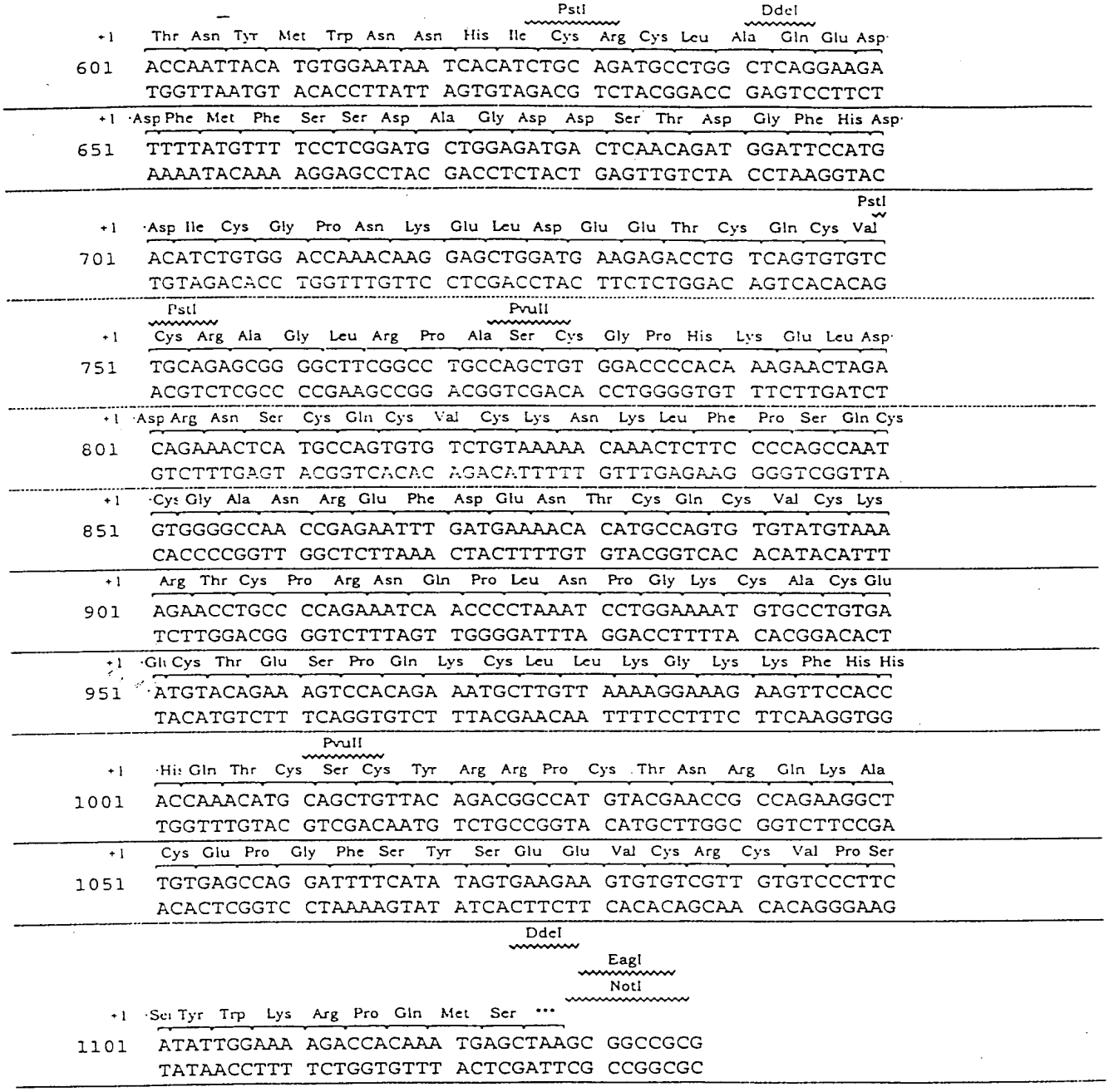
MARYANN WHITE
NOTARY PUBLIC, State of New York
No. 4883761
Qualified in Nassau County
Certification Filed in New York County
Commission Expires January 26, 2001

** TOTAL PAGE.07 **

VEGF-2 350aa +Signal Sequence:

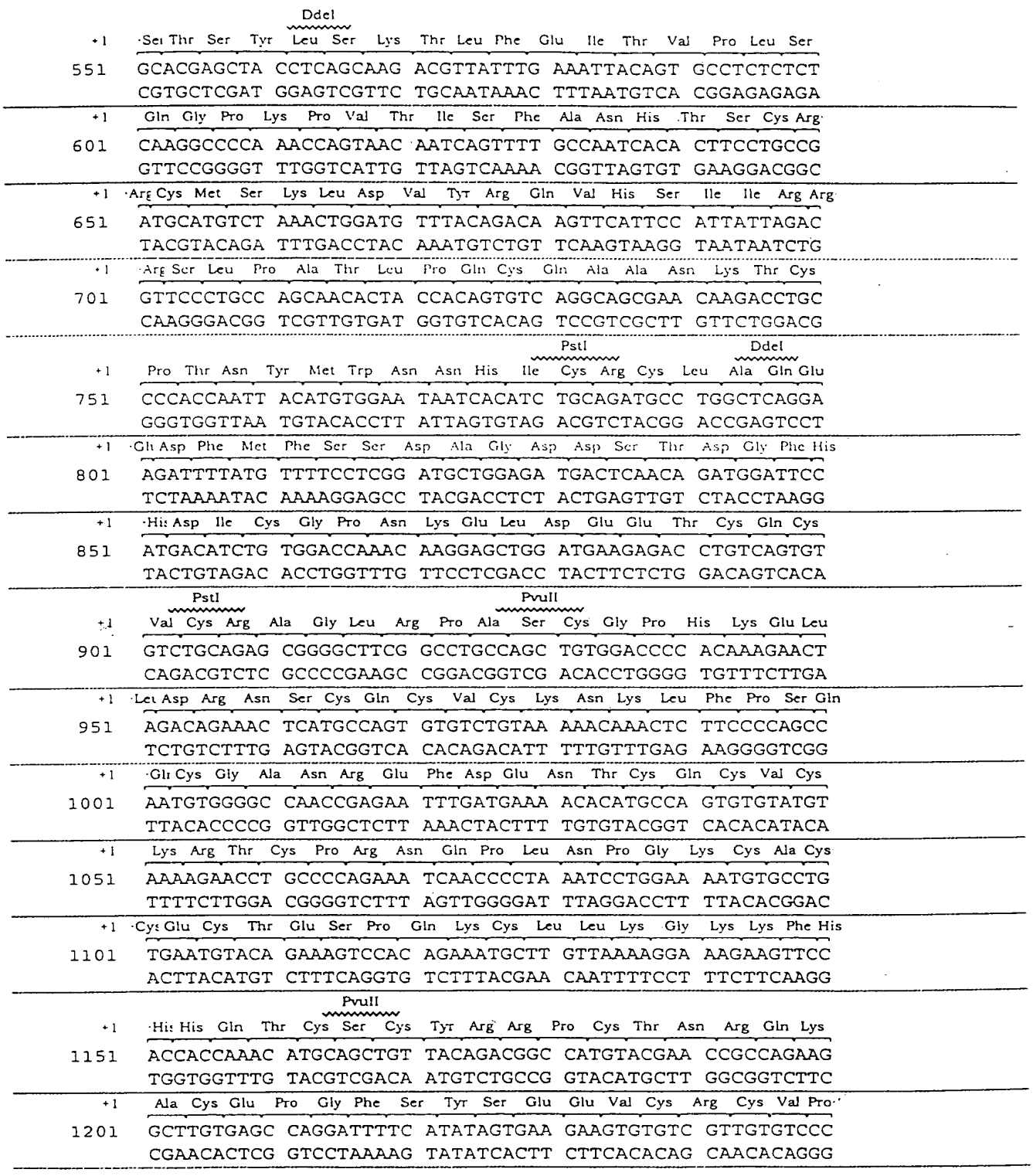


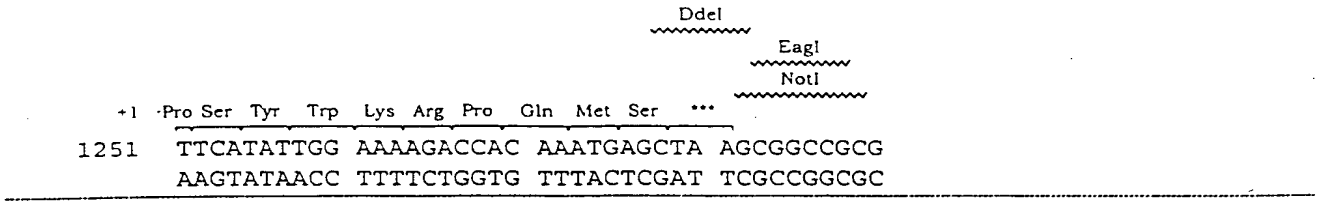
Appendix I


PstI
DdeI
+1 Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
601 ACCAATTACA TGTGGAATAA TCACATCTGC AGATGCCTGG CTCAGGAAGA
TGGTTAATGT ACACCTTATT AGTGTAGACG TCTACGGACC GAGTCCTTCT
+1 Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp
651 TTTTATGTTT TCCTCGGATG CTGGAGATGA CTCAACAGAT GGATTCCATG
AAAATACAAA AGGAGCCTAC GACCTCTACT GAGTTGTCTA CCTAAGGTAC
PstI
+1 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val
701 ACATCTGTGG ACCAAACAAG GAGCTGGATG AAGAGACCTG TCAGTGTGTC
TGTAGACACC TGGTTTGTTC CTCGACCTAC TTCTCTGGAC AGTCACACAG
PstI
PvuII
+1 Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp
751 TGCAGAGCGG GGCTTCGGCC TGCCAGCTGT GGACCCCACA AAGAACTAGA
ACGTCTCGCC CCGAAGCCGG ACGGTCGACA CCTGGGGTGT TTCTTGATCT
+1 Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys
801 CAGAAACTCA TGCCAGTGTG TCTGTAAAAA CAAACTCTTC CCCAGCCAAT
GTCTTTGAGT ACGGTCACAC AGACATTTTT GTTTGAGAAG GGGTCGGTTA
+1 Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys
851 GTGGGGCCAA CCGAGAATTT GATGAAAACA CATGCCAGTG TGTATGTAAA
CACCCCGGTT GGCTCTTAAA CTACTTTTGT GTACGGTCAC ACATACATTT
+1 Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu
901 AGAACCTGCC CCAGAAATCA ACCCCTAAAT CCTGGAAAAT GTGCCTGTGA
TCTTGGACGG GGTCTTTAGT TGGGGATTTA GGACCTTTTA CACGGACACT
+1 Gln Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His
951 ATGTACAGAA AGTCCACAGA AATGCTTGTT AAAAGGAAAG AAGTTCCACC
TACATGTCTT TCAGGTGTCT TTACGAACAA TTTTCCTTTC TTCAAGGTGG
PvuII
+1 His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala
1001 ACCAAACATG CAGCTGTTAC AGACGGCCAT GTACGAACCG CCAGAAGGCT
TGGTTTGTAC GTCGACAATG TCTGCCGGTA CATGCTTGGC GGTCTTCCGA
+1 Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser
1051 TGTGAGCCAG GATTTTCATA TAGTGAAGAA GTGTGTCGTT GTGTCCCTTC
ACACTCGGTC CTAAAAGTAT ATCACTTCTT CACACAGCAA CACAGGGAAG
DdeI
EagI
NotI
+1 Ser Tyr Trp Lys Arg Pro Gln Met Ser ***
1101 ATATTGGAAA AGACCACAAA TGAGCTAAGC GGCCGCG
TATAACCTTT TCTGGTGTTT ACTCGATTCG CCGGCGC

VEGF-2 419aa Sequence:

```
                EcoRI
                ~~~~~
+1                                  Met  His  Leu  Leu  Gly  Phe  Phe  Ser  Val  Ala
                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 1  GAATTCGTGG GTCCTTCCAC CATGCACTTG CTGGGCTTCT TCTCTGTGGC
    CTTAAGCACC CAGGAAGGTG GTACGTGAAC GACCCGAAGA AGAGACACCG

                                                SmaI
                                                XmaI
                                                AvaI          NruI
+1  Ala Cys Ser  Leu  Leu  Ala  Ala  Ala  Leu  Leu  Pro  Gly  Pro  Arg  Glu  Ala  Pro  Ala
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
51  GTGTTCTCTG CTCGCCGCTG CGCTGCTCCC GGGTCCTCGC GAGGCGCCCG
    CACAAGAGAC GAGCGGCGAC GCGACGAGGG CCCAGGAGCG CTCCGCGGGC

+1  Ala Ala  Ala  Ala  Ala  Phe  Glu  Ser  Gly  Leu  Asp  Leu  Ser  Asp  Ala  Glu  Pro
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
101 CCGCCGCCGC CGCCTTCGAG TCCGGACTCG ACCTCTCGGA CGCGGAGCCC
    GGCGGCGGCG GCGGAAGCTC AGGCCTGAGC TGGAGAGCCT GCGCCTCGGG

                                                DpnI
                                                BglII
+1  Asp  Ala  Gly  Glu  Ala  Thr  Ala  Tyr  Ala  Ser  Lys  Asp  Leu  Glu  Glu  Gln  Leu
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
151 GACGCGGGCG AGGCCACGGC TTATGCAAGC AAAGATCTGG AGGAGCAGTT
    CTGCGCCCGC TCCGGTGCCG AATACGTTCG TTTCTAGACC TCCTCGTCAA

                                                                            SspI
+1  Leu Arg  Ser  Val  Ser  Ser  Val  Asp  Glu  Leu  Met  Thr  Val  Leu  Tyr  Pro  Glu  Tyr
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
201 ACGGTCTGTG TCCAGTGTAG ATGAACTCAT GACTGTACTC TACCCAGAAT
    TGCCAGACAC AGGTCACATC TACTTGAGTA CTGACATGAG ATGGGTCTTA

    SspI                                        DdeI
+1  Tyr Trp  Lys  Met  Tyr  Lys  Cys  Gln  Leu  Arg  Lys  Gly  Gly  Trp  Gln  His  Asn
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
251 ATTGGAAAAT GTACAAGTGT CAGCTAAGGA AAGGAGGCTG GCAACATAAC
    TAACCTTTTA CATGTTCACA GTCGATTCCT TTCCTCCGAC CGTTGTATTG

                                                                            PstI
+1  Arg  Glu  Gln  Ala  Asn  Leu  Asn  Ser  Arg  Thr  Glu  Glu  Thr  Ile  Lys  Phe  Ala
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
301 AGAGAACAGG CCAACCTCAA CTCAAGGACA GAAGAGACTA TAAAATTTGC
    TCTCTTGTCC GGTTGGAGTT GAGTTCCTGT CTTCTCTGAT ATTTTAAACG

                                        DpnI
    PstI                                BglII
+1  Ala Ala  Ala  His  Tyr  Asn  Thr  Glu  Ile  Leu  Lys  Ser  Ile  Asp  Asn  Glu  Trp  Arg
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
351 TGCAGCACAT TATAATACAG AGATCTTGAA AAGTATTGAT AATGAGTGGA
    ACGTCGTGTA ATATTATGTC TCTAGAACTT TTCATAACTA TTACTCACCT

                SphI
+1  Arg Lys  Thr  Gln  Cys  Met  Pro  Arg  Glu  Val  Cys  Ile  Asp  Val  Gly  Lys  Glu
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
401 GAAAGACTCA ATGCATGCCA CGGGAGGTGT GTATAGATGT GGGGAAGGAG
    CTTTCTGAGT TACGTACGGT GCCCTCCACA CATATCTACA CCCCTTCCTC

                NruI                    DraI                            AccI
+1  Phe Gly  Val  Ala  Thr  Asn  Thr  Phe  Phe  Lys  Pro  Pro  Cys  Val  Ser  Val  Tyr
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
451 TTTGGAGTCG CGACAAACAC CTTCTTTAAA CCTCCATGTG TGTCCGTCTA
    AAACCTCAGC GCTGTTTGTG GAAGAAATTT GGAGGTACAC ACAGGCAGAT

    AccI                                                PstI
+1  Tyr Arg  Cys  Gly  Gly  Cys  Cys  Asn  Ser  Glu  Gly  Leu  Gln  Cys  Met  Asn  Thr  Ser
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
501 CAGATGTGGG GGTTGCTGCA ATAGTGAGGG GCTGCAGTGC ATGAACACCA
    GTCTACACCC CCAACGACGT TATCACTCCC CGACGTCACG TACTTGTGGT
```


DdeI
+1 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser
551 GCACGAGCTA CCTCAGCAAG ACGTTATTTG AAATTACAGT GCCTCTCTCT
CGTGCTCGAT GGAGTCGTTC TGCAATAAAC TTTAATGTCA CGGAGAGAGA
+1 Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg
601 CAAGGCCCCA AACCAGTAAC AATCAGTTTT GCCAATCACA CTTCCTGCCG
GTTCCGGGGT TTGGTCATTG TTAGTCAAAA CGGTTAGTGT GAAGGACGGC
+1 Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg
651 ATGCATGTCT AAACTGGATG TTTACAGACA AGTTCATTCC ATTATTAGAC
TACGTACAGA TTTGACCTAC AAATGTCTGT TCAAGTAAGG TAATAATCTG
+1 Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys
701 GTTCCCTGCC AGCAACACTA CCACAGTGTC AGGCAGCGAA CAAGACCTGC
CAAGGGACGG TCGTTGTGAT GGTGTCACAG TCCGTCGCTT GTTCTGGACG
PstI
DdeI
+1 Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu
751 CCCACCAATT ACATGTGGAA TAATCACATC TGCAGATGCC TGGCTCAGGA
GGGTGGTTAA TGTACACCTT ATTAGTGTAG ACGTCTACGG ACCGAGTCCT
+1 Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
801 AGATTTTATG TTTTCCTCGG ATGCTGGAGA TGACTCAACA GATGGATTCC
TCTAAAATAC AAAAGGAGCC TACGACCTCT ACTGAGTTGT CTACCTAAGG
+1 His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
851 ATGACATCTG TGGACCAAAC AAGGAGCTGG ATGAAGAGAC CTGTCAGTGT
TACTGTAGAC ACCTGGTTTG TTCCTCGACC TACTTCTCTG GACAGTCACA
PstI
PvuII
+1 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu
901 GTCTGCAGAG CGGGGCTTCG GCCTGCCAGC TGTGGACCCC ACAAAGAACT
CAGACGTCTC GCCCCGAAGC CGGACGGTCG ACACCTGGGG TGTTTCTTGA
+1 Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln
951 AGACAGAAAC TCATGCCAGT GTGTCTGTAA AAACAAACTC TTCCCCAGCC
TCTGTCTTTG AGTACGGTCA CACAGACATT TTTGTTTGAG AAGGGGTCGG
+1 Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys
1001 AATGTGGGGC CAACCGAGAA TTTGATGAAA ACACATGCCA GTGTGTATGT
TTACACCCCG GTTGGCTCTT AAACTACTTT TGTGTACGGT CACACATACA
+1 Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys
1051 AAAAGAACCT GCCCCAGAAA TCAACCCCTA AATCCTGGAA AATGTGCCTG
TTTTCTTGGA CGGGGTCTTT AGTTGGGGAT TTAGGACCTT TTACACGGAC
+1 Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His
1101 TGAATGTACA GAAAGTCCAC AGAAATGCTT GTTAAAAGGA AAGAAGTTCC
ACTTACATGT CTTTCAGGTG TCTTTACGAA CAATTTTCCT TTCTTCAAGG
PvuII
+1 His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys
1151 ACCACCAAAC ATGCAGCTGT TACAGACGGC CATGTACGAA CCGCCAGAAG
TGGTGGTTTG TACGTCGACA ATGTCTGCCG GTACATGCTT GGCGGTCTTC
+1 Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro
1201 GCTTGTGAGC CAGGATTTTC ATATAGTGAA GAAGTGTGTC GTTGTGTCCC
CGAACACTCG GTCCTAAAAG TATATCACTT CTTCACACAG CAACACAGGG


DdeI
EagI
NotI
+1 Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser ***
1251 TTCATATTGG AAAAGACCAC AAATGAGCTA AGCGGCCGCG
AAGTATAACC TTTTCTGGTG TTTACTCGAT TCGCCGGCGC

STRAIN SHEET

STRAIN CMV I STORAGE NUMBER 11:64

PEDIGREE:

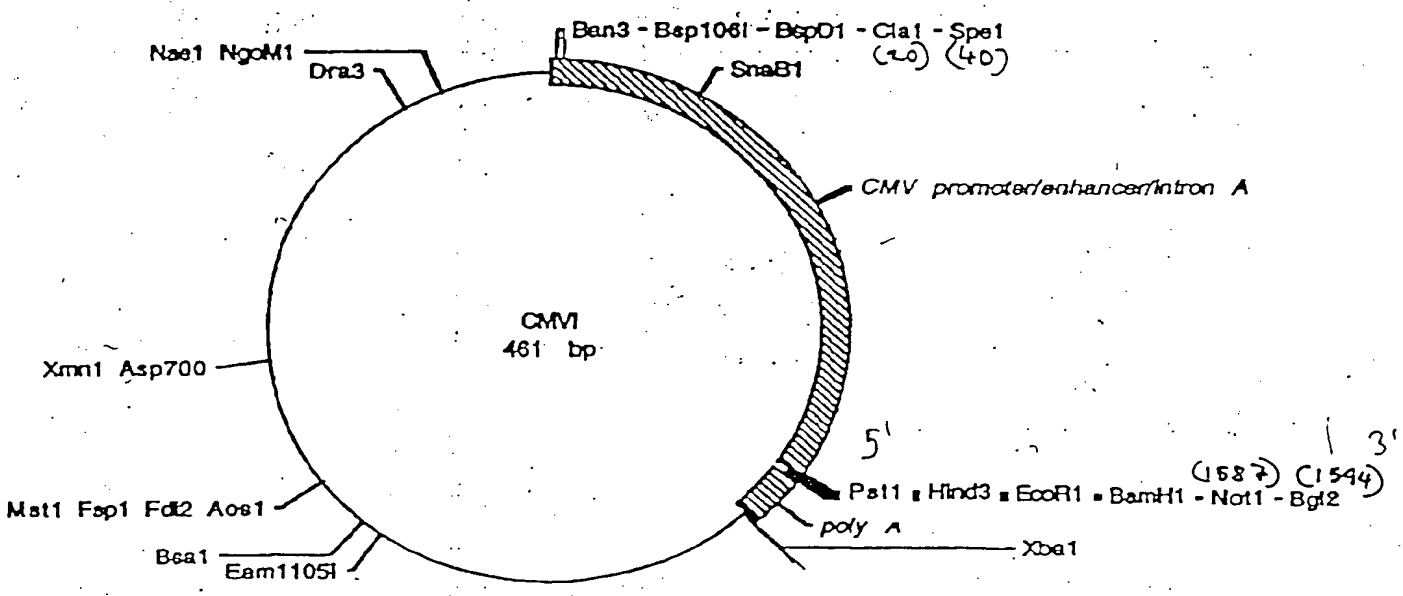
-CMVI was constructed in the pSV7 (nee pSG5 from Stratagene, with an expanded polylinker) backbone by replacing the SV40 promoter from pSV7 with the CMV promoter/enhancer/intron, via 5' Sal I/Xho I (sites destroyed) and 3' Hind III.

-CMVI is 4613 bp.

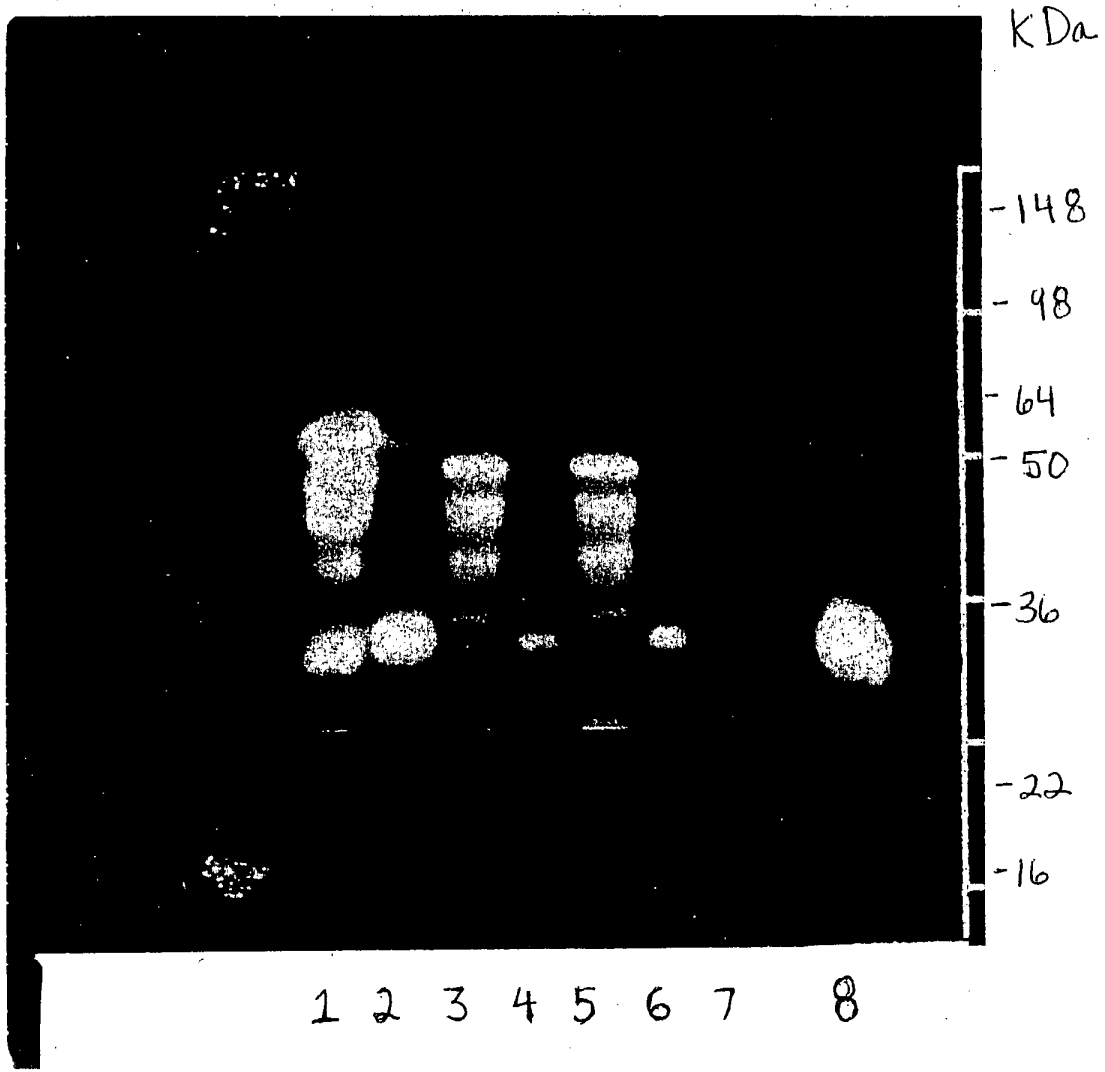
-CMVI uses ampicillin resistance.

-CMV promoter/enhancer + Intron A: nt #'s 1-1566.
-Polylinker: nt #'s 1566-1597 (5'- Hind III - Eco RI - Bam HI - Not I - Bgl II - 3').
-SV40 polyA addition sequence: nt #'s 1598-1745.
-If for some reason you want to remove the SV40 polyA addition sequence, you can cut with either Sal I or Xba I (these 2 sites border the 3' end of this sequence).

GROWTH REQUIREMENTS:

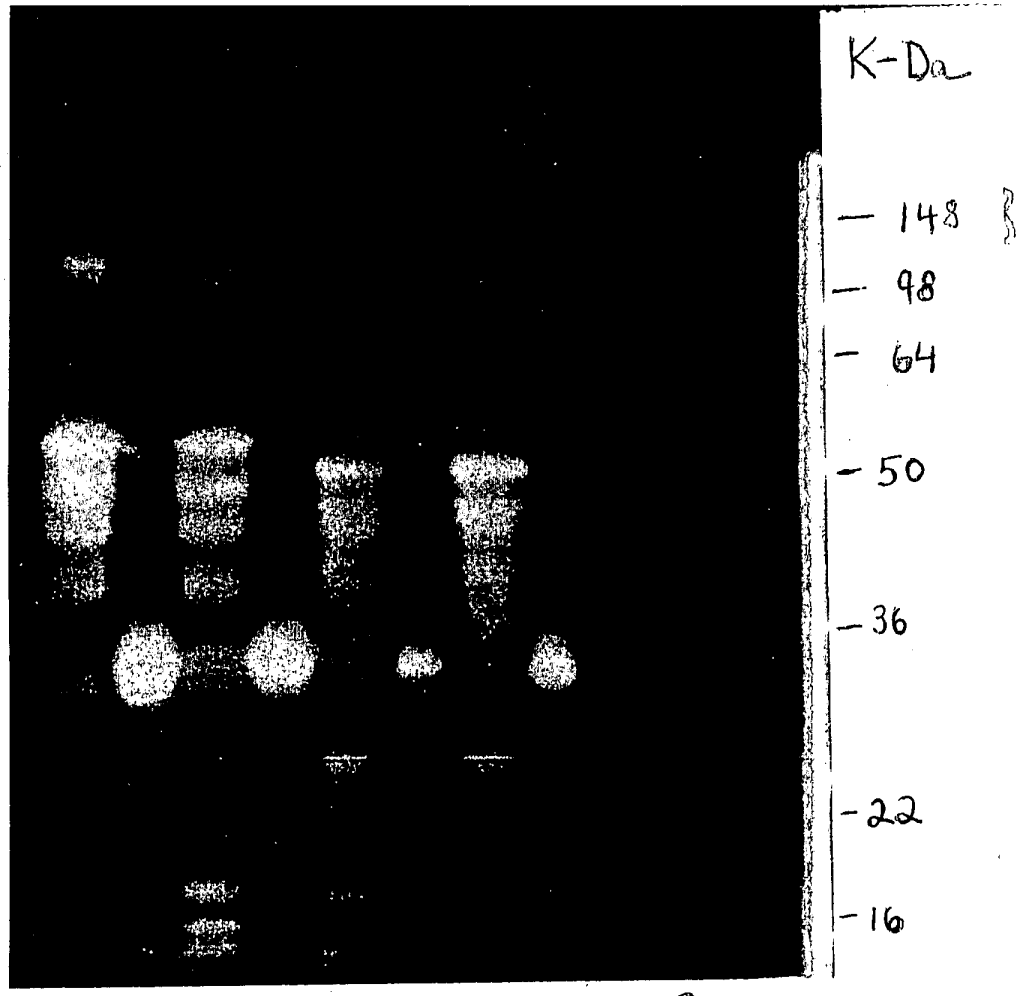


Appendix II

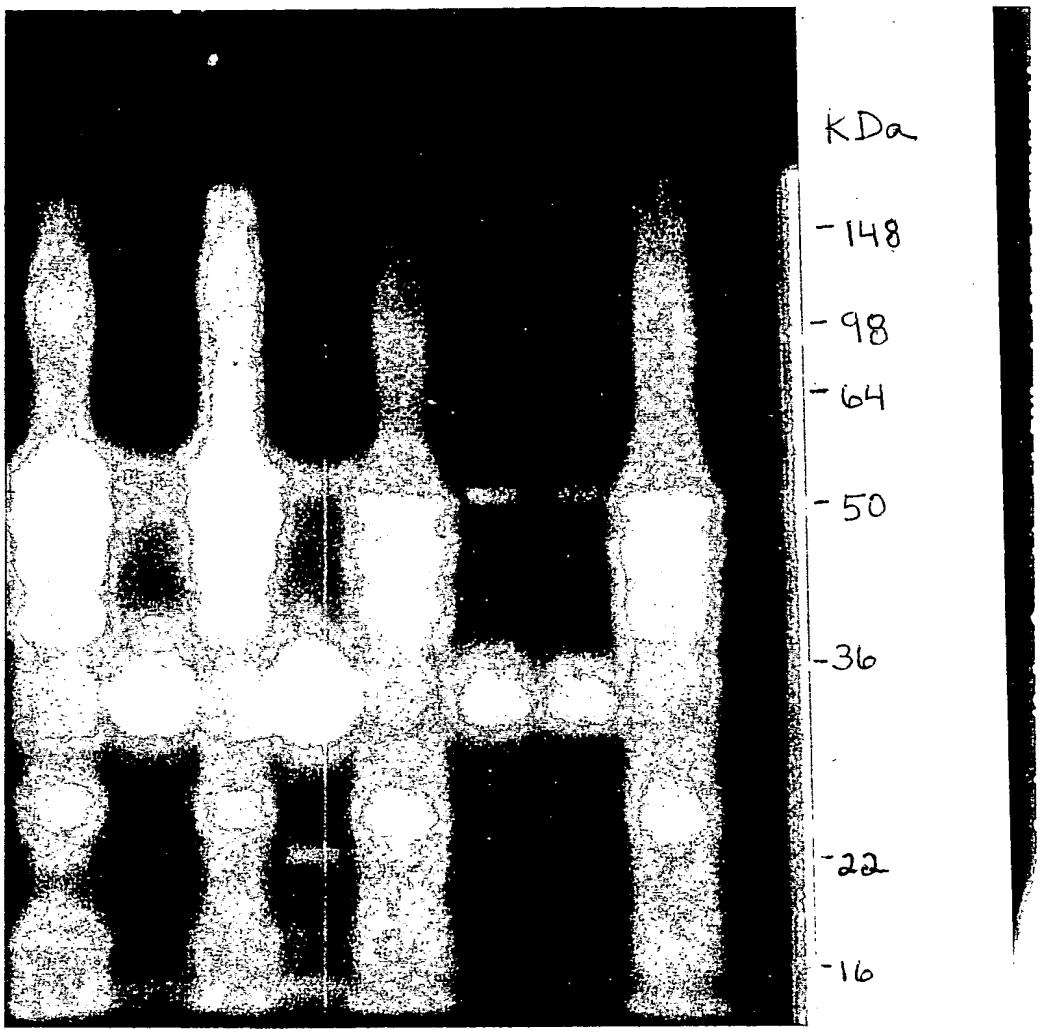


Power Declaration

Figure 1 Gel 1



Power Declaration
Figure 1 - Gel 2



Power Declaration
Figure 1 - Gel 3